

AD \_\_\_\_\_

Grant Number DAMD17-94-J-4394

TITLE: Gene Therapy of Breast Cancer: Studies of Selective Promoter/Enhancer-Modified Vectors to Deliver Suicide Genes

PRINCIPAL INVESTIGATOR: Donald W. Kufe, M.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute  
Boston, Massachusetts 02115

REPORT DATE: September 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 2

19980310 063

# REPORT DOCUMENTATION PAGE

*Form Approved  
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

<b>1. AGENCY USE ONLY (Leave blank)</b>			<b>2. REPORT DATE</b> September 1997		<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Sep 96 - 31 Aug 97)		
<b>4. TITLE AND SUBTITLE</b>  Gene Therapy of Breast Cancer: Studies of Selective Promoter/Enhancer-Modified Vectors to Deliver Suicide Genes					<b>5. FUNDING NUMBERS</b> DAMD17-94-J-4394		
<b>6. AUTHOR(S)</b>  Donald W. Kufe, M.D.							
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  Dana-Farber Cancer Institute Boston, Massachusetts 02115			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>				
<b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING/MONITORING AGENCY REPORT NUMBER</b>				
<b>11. SUPPLEMENTARY NOTES</b>							
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for public release; distribution unlimited			<b>12b. DISTRIBUTION CODE</b>				
<b>13. ABSTRACT (Maximum 200)</b>  <p>The overall goal of this project is to develop gene therapy strategies for breast cancer by translation of studies derived from the DF3/MUC1 gene. We have completed Tasks 1 and 2 as outlined in the Statement of Work using the DF3 promoter to selectively drive transgenes in breast cancer cells. The DF3 promoter has been used in an adenoviral vector to selectively detect and eliminate breast cancer cells that contaminate hematopoietic stem cell preparations used in autologous bone marrow transplantation. More recent work has involved modification of the DF3 promoter by adding a Tet-enhancer system to increase expression of the transgenes. The requisite plasmids have been constructed and are being integrated into replication-deficient adenoviral vectors to complete Tasks 3 and 4. Other studies have evaluated transduction of dendritic cells (DC) with adenoviral vectors expressing the DF3/MUC1 gene. The transduced DC are functional in primary allogeneic mixed lymphocyte reactions. Mice immunized with Ad-MUC1 transduced DC develop cytotoxic T lymphocytes specific for the DF3/MUC1 antigen. Ad-MUC1 transduced DC also induce a specific response which inhibits the growth of DF3-positive tumors. These findings support the usefulness of Ad-transduced DC for in vivo immunization against the DF3/MUC1 antigen.</p>							
<b>14. SUBJECT TERMS</b>  Breast Cancer					<b>15. NUMBER OF PAGES</b> 24		
							<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b>  Unclassified		<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b>  Unclassified		<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b>  Unclassified			<b>20. LIMITATION OF ABSTRACT</b>  Unlimited

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

DWK Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

DWK In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

DWK In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

DWK In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

DWK In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Dad w. Kafe  
PI - Signature

9/26/97  
Date

**Gene Therapy of Breast Cancer: Studies of  
Selective Promoter/Enhancer-modified  
Vectors to Deliver Suicide Genes**

Contents

Introduction.....	2
Body.....	4
Experimental Methods.....	4
Results and Discussion.....	9
Conclusions.....	12
References.....	13
Appendix.....	18

## INTRODUCTION

The overall goal of this project is to develop gene therapy strategies for the treatment of human breast cancer. We have completed Tasks 1 and 2 as outlined in our Statement of Work (SOW). Our last progress report included data demonstrating that the DF3/MUC1 promoter/enhancer can be used in an adenoviral vector to selectively detect and eliminate breast cancer cells that contaminate hematopoietic stem cell preparations used in autologous bone marrow transplantation. Our data in the mouse model as proposed in the SOW indicates that we need to enhance the level of suicide transgene expression to achieve cure of established tumors. We have therefore modified the DF3/MUC1 promoter vector by adding a TET promoter system to increase expression. In other systems, the TET promoter has enhanced transcription by 30-100-fold. The requisite DF3 promoter/TET vectors have been constructed (see Experimental Methods). These vectors are presently being integrated into replication-deficient adenoviruses before we proceed to Tasks 3 and 4.

Whereas our goal is to develop gene therapy strategies for breast cancer with a focus on the DF3/MUC1 gene, we have used our previously prepared adenoviral vectors to transduce dendritic cells and develop vaccines against the DF3 antigen. Dendritic cells (DC) are potent antigen presenting cells (APCs) that have the capacity to activate naive cytotoxic T cells (1). Murine DC pulsed with peptides prime antigen-specific CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) in vivo (2). Peptides derived from tumor-associated antigens have similarly been used to pulse DC and induce antitumor immunity (3-5). Other studies have employed

soluble tumor-associated antigens for loading DC and generating antitumor activity (6). Whereas peptides pulsed onto DC may dissociate from MHC molecules, CD34+ cells have been retrovirally transduced to stably express antigens after differentiation to DC (7,8). In contrast to pulsing, transduction of DC can result in longer-term antigen presentation and induction of immunity against undefined MHC epitopes. Thus, transduced DC may be effective in immunizing against known tumor-associated antigens.

The human DF3/MUC1 glycoprotein is aberrantly overexpressed in breast and other carcinomas (9). The DF3 protein is one member of the MUC1 family of carcinoma-associated antigens that contain variable numbers of highly conserved (G+C)-rich 60 base pair tandem repeats (10,11). A C-terminal region includes a transmembrane domain that anchors the antigen at the cell surface (12,13). Cell-cell interactions are reduced in cells transfected with the MUC1 cDNA (14). Other work has demonstrated that DF3 inhibits the recognition of targets by immune effector cells (15-17). These findings have suggested that the DF3/MUC1 tumor-associated antigen may function in inhibiting antitumor immunity.

Our recent studies demonstrate adenoviral-mediated transduction of the  $\beta$ -galactosidase and DF3/MUC1 genes in mouse DC. We also show that the transduced DC are functional in inducing antitumor immunity.

## BODY

### **Experimental Methods for DF3/MUC1 Promoter-enhancer Studies**

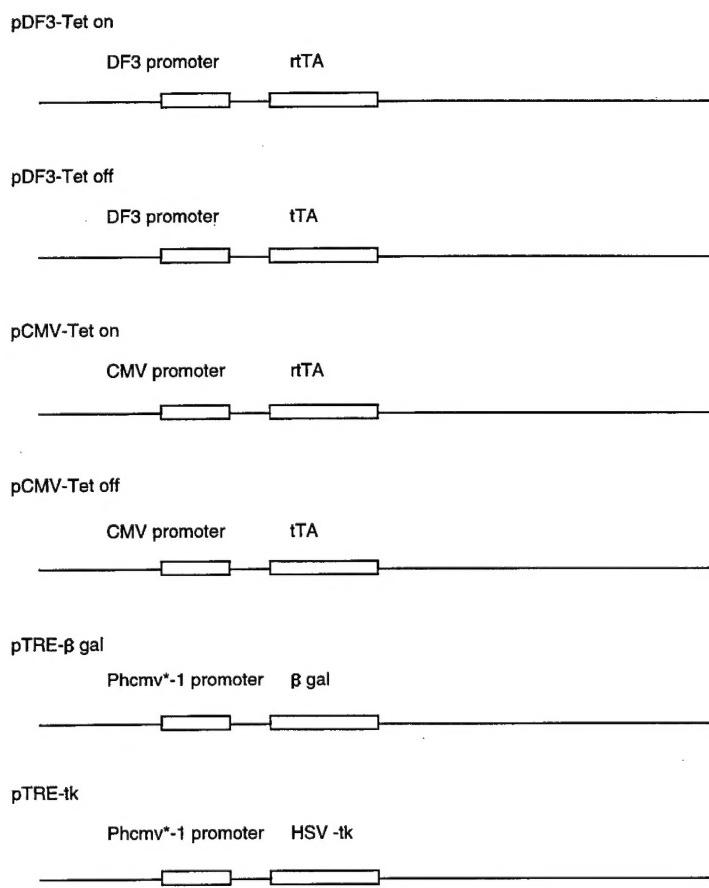
Construction of DF3 promoter/TET on/off plasmids. The rtTA gene or the tTA gene, which when expressed activate transcription of the TRE, was prepared from pTet-on or pTet-off (CLONTECH) by isolating fragments generated by digestion with BamH1 and EcoRI. The rtTA gene or the tTA gene was ligated into the EcoRV site of pDF3- $\beta$ gal by blunt end ligation. The resulting plasmids, pDF3-Tet on and pDF3-Tet off, were thus derived by replacing the lacZ gene with rtTa or tTa, respectively.

Construction of pCMV-Tet on and pCMV-Tet off plasmids. The pCMV-Tet on and the pCMV-Tet off plasmids were constructed by replacement of the lacZ gene in pCMV- $\beta$ -gal with rtTA and tTA, respectively, as described above.

Construction of pTRE- $\beta$ -gal and pTRE-tk plasmids. The Phcmv\*-1 promoter, which contains the Tet-responsive element (TRE) upstream of the minimal CMV promoter, was isolated from pTRE (CLONTECH) by digestion with EcoRI and XhoI. The Phcmv\*-1 Tet-responsive promoter was then ligated into XhoI and SpeI digested pCMV- $\beta$ gal or pCMV-tk by blunt end ligation.

The following plasmids needed to complete Tasks 3 and 4 of the SOW have been constructed and we are in the process of preparing the corresponding adenoviral (Ad) vectors. The Ad-vectors will be tested in vitro for selectivity of the DF3 promoter in DF3-positive breast cancer cells as compared to that obtained with the ubiquitously expressed CMV promoter. We will focus on the Tet on vectors because of the regulation that can be achieved by adding doxycycline in vitro or vivo in the animal

studies. The pTRE- $\beta$ gal vector will be used to assess the selectivity and potency of the pDF3-Tet on/off promoters. The pTRE-tk vector will be used in experiments designed to kill cells by expressing the suicide gene.



### **Experimental Methods for DF3/MUC1 Vaccine Studies**

Cell culture. DC were isolated from bone marrow cultures as described (18). Briefly, bone marrow flushed from the long bones of C57Bl/6 mice was treated with ammonium chloride to lyse red cells. Lymphocytes, granulocytes and Ia+ cells were depleted by incubation with monoclonal antibodies (MAbs) 2.43 (anti-CD8; ATCC, Rockville, MD), GK1.5 (anti-CD4; ATCC), RA3-3A1/6.1 (anti-B220/CD4SR; ATCC), B21.2 (anti-Ia; ATCC), RB6-85C (anti-Gr-1; Pharmingen, San Diego, CA) and rabbit complement. The cells were plated in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum, 10 mM HEPES (pH 7.4), 50 µM 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 500 U/ml recombinant murine GM-CSF (Boehringer-Mannheim). After 7 d, the tightly adherent monocytes were harvested for transduction, while the nonadherent and loosely adherent cells were collected and replated in 100-mm Petri dishes ( $8 \times 10^6$  cells/dish). The nonadherent cells were removed after 30 min by washing, and medium containing GM-CSF was added to the dish. The cells were incubated for 18-24 h and the floating DC population was then harvested for analysis and transduction.

Recombinant adenoviral infection. Ad.βgal and Ad.MUC1 are structurally similar replication-deficient recombinant adenoviruses in which the lacZ and DF3/MUC1 genes (19), respectively, are under control of the CMV immediate-early promoter and enhancer (20,21). DC and monocytes were incubated with recombinant adenovirus at the indicated multiplicity of infection (MOI) for 6 h, washed and then cultured in medium containing GM-CSF.

Analysis of adenoviral-transduced DC. Cells were washed with PBS and incubated with MAb D19-2F3-2 (anti- $\beta$ gal; Boehringer-Mannheim), DF3 (anti-MUC1), M1/42/3.9.8 (anti-MHC Class I; ATCC), M5/114 (anti-MHC Class II; ATCC), 16-10A1 (anti-B7-1; provided by Dr. Hans Reiser, Dana-Farber), GL1 (anti-B7-2; Pharmingen) or 3E2 (anti-ICAM; Pharmingen) for 30 min on ice. After washing, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-hamster, -rat or -mouse IgG for analysis by FACScan (Becton-Dickinson). For immunoperoxidase staining, the cells were centrifuged onto slides, incubated with MAb DF3 or MAb M5/114 and stained by the avidin-biotin complex method (Vector Laboratories, Inc., Burlingame, CA) (22).

Mixed lymphocyte reactions. Control and transduced DC were treated with 20 Gy ionizing radiation. The cells were incubated at varying ratios with syngeneic (C57Bl/6) or allogeneic (Balb/c) T cells in 96-well flat-bottomed plates for 4-5 d. The T cells were prepared by passing spleen suspensions through nylon wool columns, incubation for 90 min in culture dishes and collection of the nonadherent cells. Stimulation of T cells was assessed by pulsing with 1  $\mu$ Ci/well [ $^3$ H]thymidine (New England Nuclear) for 6 h and monitoring for tritium incorporation.

Immunoblot analysis. Lysates from control and Ad.MUC1-transduced DC were subjected to electrophoresis in 6% polyacrylamide gels and analysis for reactivity with MAbs DF3 and DF3-P as described (23).

Immunizations. C57Bl/6 mice were injected intravenously with  $5 \times 10^5$  DC or monocytes on day 0 and again on day 10.

CTL assays. CTL activity was determined by the lactate dehydrogenase (LDH) release assay (CytoTox, Promega, Madison, WI) (24). Splenocytes isolated from mice were subjected to Ficoll density gradient centrifugation. The splenocytes were incubated with target cells at varying E:T ratios in V bottom microtitration plates (Nunc;, Roskilde, Denmark), centrifuged for 3 min at 1000g and incubated for 4 h at 37°C. At the end of coculturing, 50 µl supernatant were transferred to an assay plate and incubated with 50 µl of substrate mixture for 30 min at room temperature. Absorbance was determined at 490-429 nm by microplate reader (Model 3550, BIO-Rad Laboratories, CA). Killing of target cells by effectors was determined by the formula: Cytotoxicity (%)=100 x (experiment release - spontaneous release)/(maximum release - spontaneous release).

Antitumor activity. Mice were immunized twice (day 0 and 10) by intravenous injection of  $5 \times 10^5$  DC or monocytes. On day 18, mice were challenged subcutaneously with  $2 \times 10^5$  MC-38 cells that stably express DF3/MUC1 (19). Tumors >3mm or greater in diameter as determined by vernier callipers were scored as positive.

## RESULTS AND DISCUSSION

Flow cytometry was used to define the phenotype of DC following transduction with recombinant adenovirus. DC derived from bone marrow expressed MHC class I and II products, costimulatory molecules and ICAM-1 (18) (Fig. 1A). Transduction with Ad. $\beta$ gal resulted in a similar pattern of antigen expression (Fig. 1A). Moreover, transduction with Ad.MUC1 was associated with DF3/MUC1 expression and little if any effect on cell surface levels of MHC, costimulatory or adhesion molecules (Fig. 1A). The Ad.MUC1-transduced DC exhibited a typical morphology with veiled dendrites (Fig. 1B). Staining with MAb M5/114 (anti-MHC class II) and MAb DF3 demonstrated expression of DF3/MUC1 by the transduced DC (Fig. 1B). Immunoblot analysis of the Ad.MUC1 transduced DC confirmed DF3/MUC1 expression (Fig. 1C). Whereas MAb DF3 detects glycosylated MUC1, the finding that MAb DF3-P reacts with a ~55 kD protein in the transduced DC also provides support for detection of the unglycosylated protein core (23) (Fig. 1C).

DC are potent stimulators of primary mixed lymphocyte reactions (MLR) (25,26). To assess in part the function of Ad-transduced DC, we compared their effects in primary allogeneic MLR with that obtained from non-transduced DC. The results demonstrate that DC transduced with Ad.MUC1 or Ad. $\beta$ gal at an MOI of 100 exhibit the same potent stimulatory function as control DC (Fig. 2A). By contrast, DC transduced at MOIs of 200 or 500 exhibited decreases in viability (data not shown) and in T cell stimulation (Fig. 2B). These results indicate that expression of adenoviral, rather than the transgene, proteins is responsible for the loss of DC function.

To determine whether Ad-transduced DC induce antitumor immunity, we immunized mice twice with uninfected DC, Ad.MUC1-transduced DC or Ad. $\beta$ gal-transduced DC. Splenocytes were assayed for CTL activity using as targets syngeneic MC-38 carcinoma cells that stably express DF3/MUC1 (19). T cells from mice immunized with Ad.MUC1-transduced DC exhibited strong activity against MC-38/MUC1, but not wild-type MC-38, cells (Fig. 3A). CTLs from these mice also induced lysis of Ad.MUC1-, and not Ad. $\beta$ gal-transduced, MC-38 cells (Fig. 3A). By contrast, T cells from mice immunized with Ad. $\beta$ gal-transduced DC exhibited lysis of only the Ad. $\beta$ gal-transduced MC-38 cells (Fig. 3B). These findings indicated that Ad-transduced DC induce immunity which is directed against the transgene.

Incubation of CTLs from mice immunized with Ad.MUC1-transduced DC with anti-CD4 or anti-CD8 antibodies blocked lysis of the MC-38/MUC1 targets (Fig. 4A). These results indicated that Ad.MUC1-transduced DC generate MHC class I and II-restricted T cell responses. The finding that incubation of MC-38/MUC1 targets with MAb DF3 blocks lysis provided further support for specificity against DF3/MUC1 (Fig. 4A). Moreover, incubation of YAC-1 cells with the CTLs showed no specific lysis (data not shown).

Immunization of mice with Ad.MUC1-transduced DC also inhibited growth of MC-38/MUC1 tumors, while Ad. $\beta$ gal-transduced or non-transduced DC had no effect on tumor growth (Fig. 4B). To assess the potency of the Ad-transduced DC, we compared induction of immunity with that obtained when using monocytes. The efficiency of Ad. $\beta$ gal- and Ad.MUC1-mediated transduction of DC and monocytes was similar (data not shown). However, immunization with Ad.MUC1-

transduced monocytes was less effective than Ad.MUC1-transduced DC in the induction of CTL activity (Fig. 4C). Ad.MUC1-transduced monocytes were also less effective than the transduced DC in inhibiting the growth of MC38/MUC1 tumors (Fig. 4D). These findings are in concert with the greater potency of DC as APCs (1).

Previous studies have demonstrated retroviral transduction of human CD34<sup>+</sup> progenitor cells and then differentiation of the transduced cells into DC by cytokine stimulation (7,8). The differentiated DC expressed the transgene and were functional in stimulating T cells in vitro (7,8). Whereas retroviral transduction requires proliferating cells, adenoviral-transduced gene expression is not dependent on cell growth. The present studies demonstrate that murine DC can be efficiently transduced by adenoviral vectors. Transduction of DC with Ad.MUC1 or Ad. $\beta$ gal at an MOI of 100 resulted in over 80% of the cells expressing the transgene. Similar transduction efficiencies were obtained in monocytes and fibroblasts. Whereas transduction at an MOI of 100 had no effect on stimulation in the MLR assay, higher MOIs (200 and 500) resulted in lower levels of T cell proliferation. This finding was associated with cytopathic effects observed at the higher MOIs.

Studies with retrovirally transduced CD34<sup>+</sup> cells that differentiate to DC have not been performed in an animal model; therefore, it is not known whether these cells are useful for in vivo immunization. The present studies demonstrate that immunization with Ad.MUC1- or Ad. $\beta$ gal-transduced mouse DC induce CTL responses that are specific for the transgene. Reactivity

against adenoviral antigens was apparently low based on the selectivity of the CTL response against DF3/MUC1 or  $\beta$ gal. Treatment of the CTLs with antibodies against T cell subsets indicated that the Ad-transduced DC stimulate a CD4 $^{+}$  and CD8 $^{+}$  immune response.

## **CONCLUSIONS**

Induction of anti-DF3/MUC1 immunity with the Ad.MUC1-transduced DC was sufficient to specifically inhibit the growth of DF3/MUC1-positive tumor cells. These findings support the usefulness of Ad-transduced DC for in vivo immunization against tumor-associated antigens.

## REFERENCES

1. Steinman, R. M. The dendritic cell system and its role in immunogenicity. *Ann. Rev. Immunol.*, 9:271-296, 1991.
2. Porgador, A., and Gilboa, E. Bone marrow-generated dendritic cells pulsed with a class I-restricted peptide are potent inducers of cytotoxic T lymphocytes. *J. Exp. Med.*, 182:255-260, 1995.
3. Mayordomo, J. I., Zorina, T., Storkus, W. J., Zitvogel, L., Celluzzi, C., Falo, L. D., Melief, C. J., Ildstad, S. T., Kast, W. M., Deleo, A. B., and Lotze, M. T. Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nature Med.*, 1:1297-1302, 1995.
4. Celluzzi, C. M., Mayordomo, C. I., Storkus, W. J., Lotze, M. T., and Falo, L. D. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. *J. Exp. Med.*, 183:283-287, 1996.
5. Zitvogel, L., Mayordomo, J. I., Tjandrawan, T., DeLeo, A. B., Clarke, M. R., Lotze, M. T., and Storkus, W. J. Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells. *J. Exp. Med.*, 183:87-97, 1996.
6. Paglia, P., Chiodoni, C., Rodolfo, M., and Colombo, M. P. Murine dendritic cells loaded in vitro with soluble protein prime cytotoxic T lymphocytes against tumor antigen in vivo. *J. Exp. Med.*, 183:317-322, 1996.
7. Reeves, M. E., Royal, R. E., Lam, J. S., Rosenberg, S. A., and Hwu, P. Retroviral transduction of human dendritic cells

- with a tumor-associated antigen gene. *Cancer Research*, 56:5672-5677, 1996.
8. Henderson, R. A., Nimgaonkar, M. T., Watkins, S. C., Robbins, P. D., Ball, E. D., and Finn, O. J. Human dendritic cells genetically engineered to express high levels of the human epithelial tumor antigen mucin (MUC-1). *Cancer Res.*, 56:3763-3770, 1996.
9. Kufe, D., Inghirami, G., Abe, M., Hayes, D., Justi-Wheeler, H., and Schlom, J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma*, 3:223-232, 1984.
10. Gendler, S., Taylor-Papadimitriou, J., Duhig, T., Rothbard, J., and Burchell, J. A. A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. *J. Biol. Chem.*, 263:12820-12823, 1988.
11. Siddiqui, J., Abe, M., Hayes, D., Shani, E., Yunis, E., and Kufe, D. Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma-associated antigen. *Proc. Natl. Acad. Sci. USA*, 85:2320-2323, 1988.
12. Ligtenberg, M. J. L., Vos, H. L., Gennissen, A. M. C., and Hilkens, J. Episialin, a carcinoma-associated mucin, is generated by a polymorphic gene encoding splice variant with alternative amino termini. *J. Biol. Chem.*, 265:5573-5578, 1990.
13. Merlo, G. R., Siddiqui, J., Cropp, C., Liscia, D. S., Lidereau, R., Callahan, R., and Kufe, D. Frequent alteration

- of the DF3 tumor-associated antigen gene in primary human breast carcinomas. *Cancer Res.*, 49:6966-6971, 1989.
14. Ligtenberg, M. J. L., Buijs, F., Vos, H. L., and Hilkens, J. Suppression of cellular aggregation by high levels of episialin. *Cancer Res.*, 52:2318-2324, 1992.
15. Hayes, D. F., Silberstein, D. S., Rodrigue, S., and Kufe, D. W. DF3 antigen, a human epithelial cell mucin, inhibits adhesion of eosinophils to antibody-coated targets. *J. Immunol.*, 145:962-970, 1990.
16. van de Wiel-van Kemenade, E., Ligtenberg, M. J. L., de Boer, A. J., Buijs, F., Vos, H. L., Melief, C. J. M., Hilkens, J., and Figdor, C. G. Episialin (MUC1) Inhibits Cytotoxic Lymphocyte-Target Cell Interaction. *The Journal of Immunology*, 151:767-776, 1993.
17. Gimmi, C. D., Morrison, B. W., Mainprice, B. A., Boussiotis, V. A., Gribben, J. G., Freeman, G. J., Hayes, D. F., Park, S. L., Kufe, D. W., and Nadler, L. M. Breast cancer associated antigen, DF3/MUC1, induces apoptosis of activated human T cells. *Nature Medicine*, 2:1367-1370, 1996.
18. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., and Steinman, R. M. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.*, 176:1693-1702, 1992.
19. Akagi, J., Hodge, J. V., McLaughlin, J. P., Fritz, L., Panicali, D., Kufe, D., J., S., and Kantor, J. A. Therapeutic antitumor response after immunization with an admixture of recombinant vaccinia viruses expressing a modified MUC1 gene

- and the murine T-cell costimulatory molecule B7. J. Immunotherapy, 20:38-47, 1997.
20. Chen, L., Chen, D., Manome, Y., Dong, Y., Fine, H. A., and Kufe, D. W. Breast cancer selective gene expression and therapy mediated by recombinant adenoviruses containing the DF3/MUC1 promoter. J. Clin. Invest., 96:2775-2782, 1995.
21. Chen, L., Pulsipher, M., Chen, D., Sieff, C., Elias, A., Fine, H. A., and Kufe, D. W. Selective transgene expression for detection and elimination of contaminating carcinoma cells in hematopoietic stem cell sources. J. Clin. Invest., 98:2539-2548, 1996.
22. Gong, J. L., McCarthy, K. M., Telford, J., Tamatani, T., Miyasaka, M., and Schneeberger, E. E. Intraepithelial airway dendritic cells: A distinct subset of pulmonary dendritic cells obtained by microdissection. J. Exp. Med., 172:797-807, 1992.
23. Perey, L., Hayes, D. F., Maimonis, P., Abe, M., O'Hara, C., and Kufe, D. W. Tumor selective reactivity of a monoclonal antibody prepared against a recombinant peptide derived from the DF3 human breast carcinoma-associated antigen. Cancer Res., 52:2563-3568, 1992.
24. Franke, L., and Porstman, T. A highly sensitive non-radioactive cytotoxicity assay for human target cells. J. Immunol. Methods, 171:259-262, 1994.
25. Steinman, R. M., and Witmer, M. D. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. Proc. Natl. Acad. Sci. U.S.A., 75:5132-5136, 1978.

26. van Voorhis, W. C., Valinksy, J., Hoffman, E., Luban, J.,  
Hair, L. S., and Steinman, R. M. Relative efficacy of human  
monocytes and dendritic cells as accessory cells for T cell  
replication. J. Exp. Med., 158:174-191, 1983.

## APPENDIX

Figure 1. Adenoviral-mediated transduction of DC. A. DC were transduced with Ad. $\beta$ gal or Ad.MUC1 at an MOI of 100. Non-transduced and transduced DC were analyzed by flow cytometry for the indicated antigens (solid areas). The open areas represent staining with control antibodies. B. Cytocentrifuge preparations of control (uninfected) and Ad.MUC1-transduced DC were reacted with MAbs M5/114 (anti-Ia; blue color) and MAbs DF3 (anti-MUC1; red color). C. Lysates from control and Ad.MUC1-transduced DC were analyzed by immunoblotting with MAbs DF3 and DF3-P.

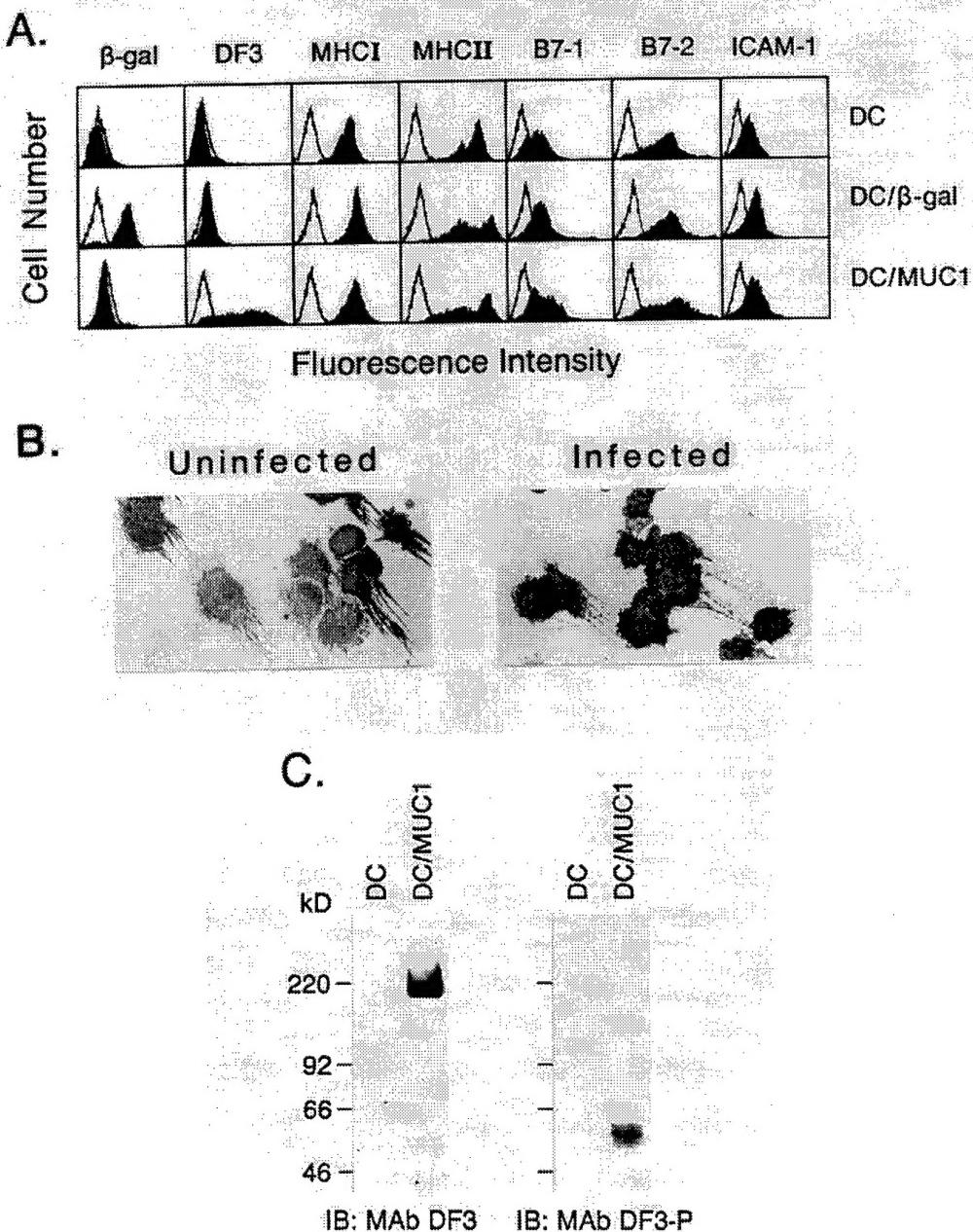


Figure 2. Induction of T cell proliferation responses with adenoviral-transduced DC. A. Non-transduced DC (solid bars) and DC transduced (MOI=100) with Ad. $\beta$ gal (diagonal bars) or Ad.MUC1 (horizontal bars) were irradiated and then incubated at the indicated ratios with  $2 \times 10^5$  allogeneic Balb/c T cells. The cells were cocultured in the MLR for 5 d. [ $^3$ H]thymidine uptake was assessed for 6 h at the end of coculturing. The results are expressed as the mean  $\pm$  SE of three experiments each performed in triplicate. B. Non-transduced DC ( $\bullet$ ) and DC transduced with Ad.MUC1 at MOIs of 100, 200, and 500 ( $\circ$ ) were incubated with  $1 \times 10^5$  Balb/c T cells. The results are expressed as the mean  $\pm$  SE of three experiments each performed in triplicate.

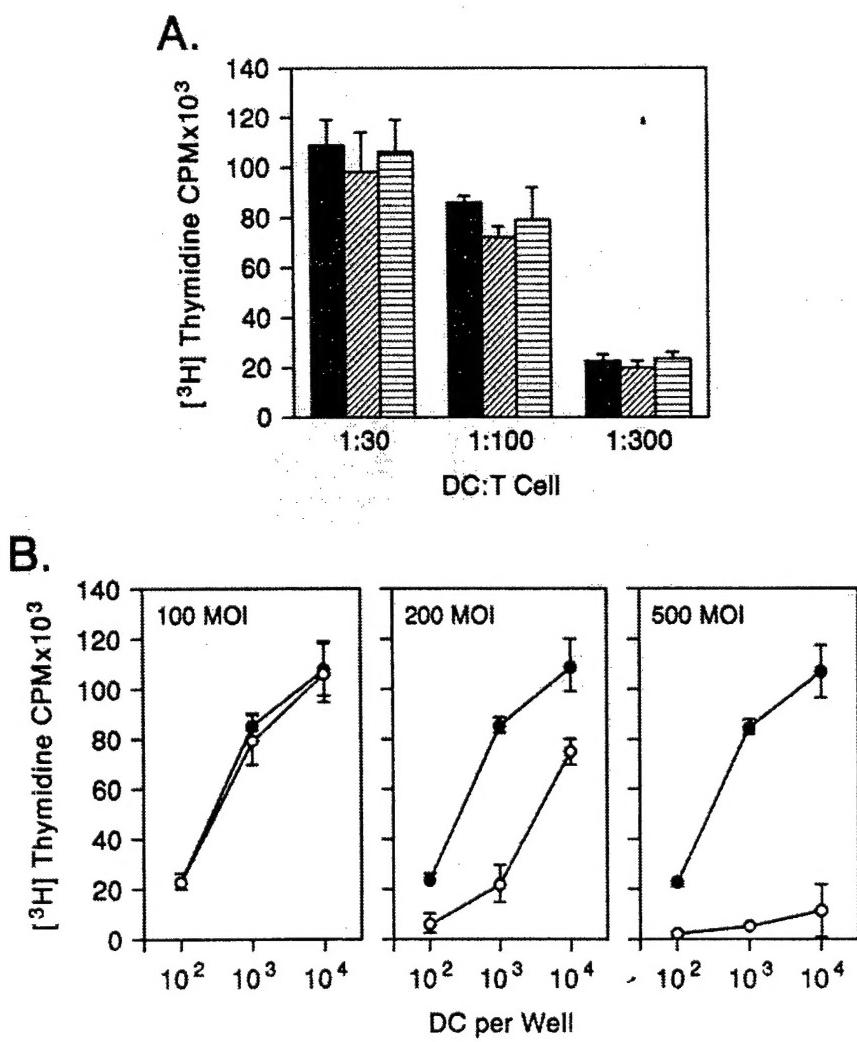
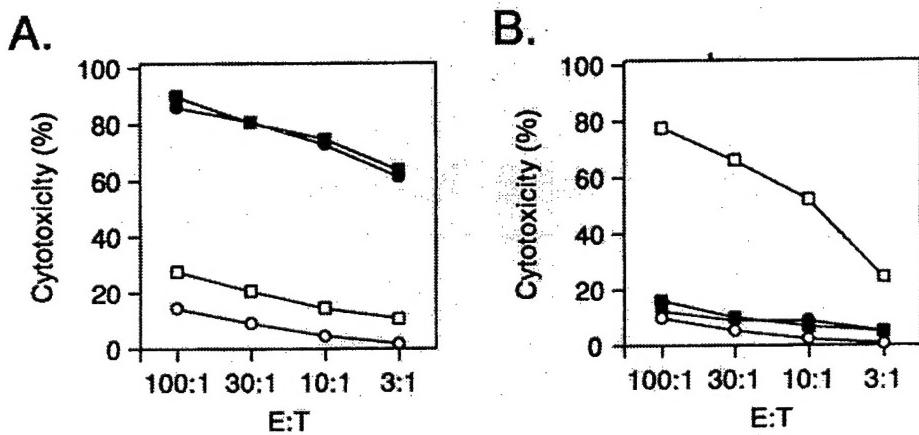


Figure 3. CTL activity of mice immunized with adenoviral-transduced DC. A and B. C57Bl/6 mice were immunized with  $5 \times 10^5$  Ad.MUC1- (A) or Ad. $\beta$ gal-transduced (B) DC on days 1 and 10. Splenocytes isolated on day 20 were incubated with MC-38 (○), MC-38/MUC1 (●), Ad.MUC1-transduced MC-38 (■) or Ad- $\beta$ gal-transduced MC-38 (□) cells at the indicated ratios. CTL activity was determined by the 4 h LDH release assay. The results are expressed as percentage cytotoxicity determined from three experiments each performed in triplicate.



**Figure 4.** Antitumor activity of mice immunized with adenoviral-transduced DC or monocytes. A. Mice were immunized twice with  $5 \times 10^5$  Ad.MUC1-transduced DC. Splenocytes isolated on day 20 were incubated with no antibody (●), rat IgG (○), anti-CD4 (▲) or anti-CD8 (■) for 1 h at 4° C. Splenocytes were incubated with MC-38/MUC1 cells at the indicated ratios. MC-38/MUC1 cells were also incubated with 25 µg/ml MAb DF3 and then mixed with splenocytes otherwise not exposed to antibody (□). CTL activity was determined by the 4 h LDH release assay for three experiments each performed in triplicate. B. Groups of 10 mice were immunized twice with  $5 \times 10^5$  DC (Δ), Ad.MUC1-transduced DC (●) or Ad.βgal-transduced DC (○). The mice were then challenged with subcutaneous injections of  $2 \times 10^5$  MC-38/MUC1 tumor cells. Tumor growth >3 mm in diameter was scored as positive. Similar results were obtained in four separate experiments. C. Mice were immunized twice with  $5 \times 10^5$  DC (○), Ad.MUC1-transduced DC (●) or Ad.MUC1-transduced monocytes (▲). Splenocytes were incubated with MC-38/MUC1 cells at the indicated ratios. CTL activity was determined by the 4 h LDH release assay for three experiments each performed in triplicate. D. Groups of 10 mice were immunized twice with  $5 \times 10^5$  DC (○), Ad.MUC1-transduced DC (●) or Ad.MUC1-transduced monocytes (▲). The mice were then challenged with subcutaneous injections of  $2 \times 10^5$  MC-38/MUC1 cells. Tumor growth >3 mm in diameter was scored as positive.

